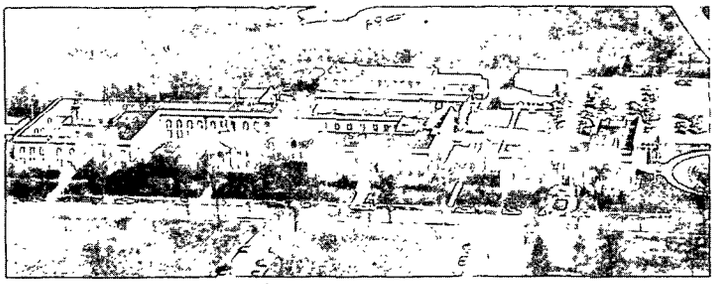

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DIFFERENTIATION OF TANNIN, LIPID, AND STARCH IN CULTURED PLANT CELLS

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INTRODUCTION

The work described herein represents a microtechnique being applied in Institute Project 3223, The Mass Production of Conifer Tree Hybrids. By utilizing this staining procedure, the correlation of observations made by light and electron microscopy of the same cells and tissues is more readily facilitated. Tannin, lipid, and starch often appear as major inclusions of the experimental cells used in this research.

This paper is being submitted for publication in Stain Technology.

ABSTRACT

Callus and cell suspension cultures of Pseudotsuga menziesii and Pinus taeda sampled for electron microscopy were fixed in 2% glutaraldehyde: 2% acrolein and then 1% OsO_4, embedded in a low-viscosity epoxy, sectioned at 0.5 µm, and stained on a glass slide with 0.3% Sudan Black B in 70% ethanol for 1 hour at 60°C. Vacuolar tannins stained brownish orange while lipid bodies turned very dark blue to black. Starch grains and cell walls were seen almost white against the blue-gray hue of the stained epoxy matrix. The observed polychromasia was confirmed by electron microscopy of the same embedment areas.

In ultrastructural studies of callus and cell suspensions of two conifer species under different culture conditions, we have noted marked variability in the amounts of storage starch and lipid and especially in the accumulation of vacuolar tannins. Correlation of such electron microscopic detail with that gained from stained light microscope sections of the same cells has given us better insight into the general physiological and organizational state of tissues composed of or arising from these cells. Numerous staining procedures for epoxy-embedded plant tissues were tried (Feder and O'Brien 1968, Kosakai 1973, Croley et al. 1973, Spurlock et al. 1966, Ledingham and Simpson 1970), but Sudan Black B as instituted by Bronner (1975) proved the most useful for clarifying the presence and distribution of these ergastic products.

MATERIALS AND METHODS

Sample Preparation: Small pieces of callus and cells/cell clusters from suspension cultures of Pseudotsuga menziesii (Mirb.) Franco and Pinus taeda L. were fixed in centrifuge tubes at 4°C for 12 hr in a 1:1 mixture of 2% glutaraldehyde:2% acrolein in sodium cacodylate buffer (0.05M at pH 7.0). After several rinses, a soak, and gentle centrifugation in buffer, the samples were postfixed at 4°C for 12 hr in buffered 1% OsO_4. Several more rinses in buffer preceded a graded acetone series at room temperature, which was followed by infiltration and embedment in a "firm" formulation, low-viscosity epoxy (Spurr 1969). Sectioning was carried out on a Sorvall MT-2B ultramicrotome equipped with a diamond knife. For light microscopy, semithin sections about 0.5 μm in thickness were flattened and dried on glass slides for 3 min at 75°C. Ultrathin sections were cut from the same embedment regions for electron microscopy.

Staining: With the epoxy matrix in place, the semithin sections were stained with Sudan Black B (Allied Chemical) according to the schedule of Bronner (1975) as adapted from the procedures of Lison (1960) and Martoja (1967). A saturated solution (about 0.3%) of stain was prepared fresh in 70% ethanol and allowed to age in an oven for 12 hr at 37°C in a vented dropper bottle. After it was filtered, the stain was warmed again in a covered Coplin jar for 30 min at 60°C just before use. The slides with the semithin sections were placed in the Coplin jar in a water bath at 60°C, covered, and stained for 1 hr. They were then rinsed in 70% ethanol and washed briefly in water. The sections were then mounted under No. 1-1/2 cover slips with Karo syrup.

Sections for electron microscopy were stained sequentially with uranyl acetate and lead citrate.

Microscopy: Observations and photomicrographs of the stained semithin sections were made with an Olympus BHA microscope using brightfield and phase optics. With the latter, better contrast and resolution were obtained, and the tinctorial differences resulting from the staining procedure were essentially unaltered.

RESULTS

By this staining method, tannin accumulations are seen as brownish orange globules in the larger cell vacuoles and can also be detected as small deposits in vacuoles developing from the rough endoplasmic reticulum (Fig. 1). Tannins are osmiophilic and thus appear black in the transmission electron micrographs (TEM) (Fig. 2). Lipids in the TEM photos are dark gray and correspond to the very dark blue to black deposits in the Sudan Black-stained sections. In

the latter, starch grains remain unstained, and they, in addition to the cell walls, are seen as very light regions against the blue-gray hue of the stained epoxy matrix. In general, plastids and nuclei also stain blue-gray and nucleoli a shade darker.

COMMENTS

This staining technique has been demonstrated to be specific for plant lipids and starch (see Bronner 1975), but the present paper illustrates its further utility in rendering plant tannins tinctorially different from these other two types of plant cell ergastics. We have found the method especially beneficial for screening sections of cultured plant cells in which starch, lipid, and tannins may represent major inclusions (Winton et al. 1974, Chafe and Durzan 1973, Baur and Walkinshaw 1974).

ACKNOWLEDGMENT

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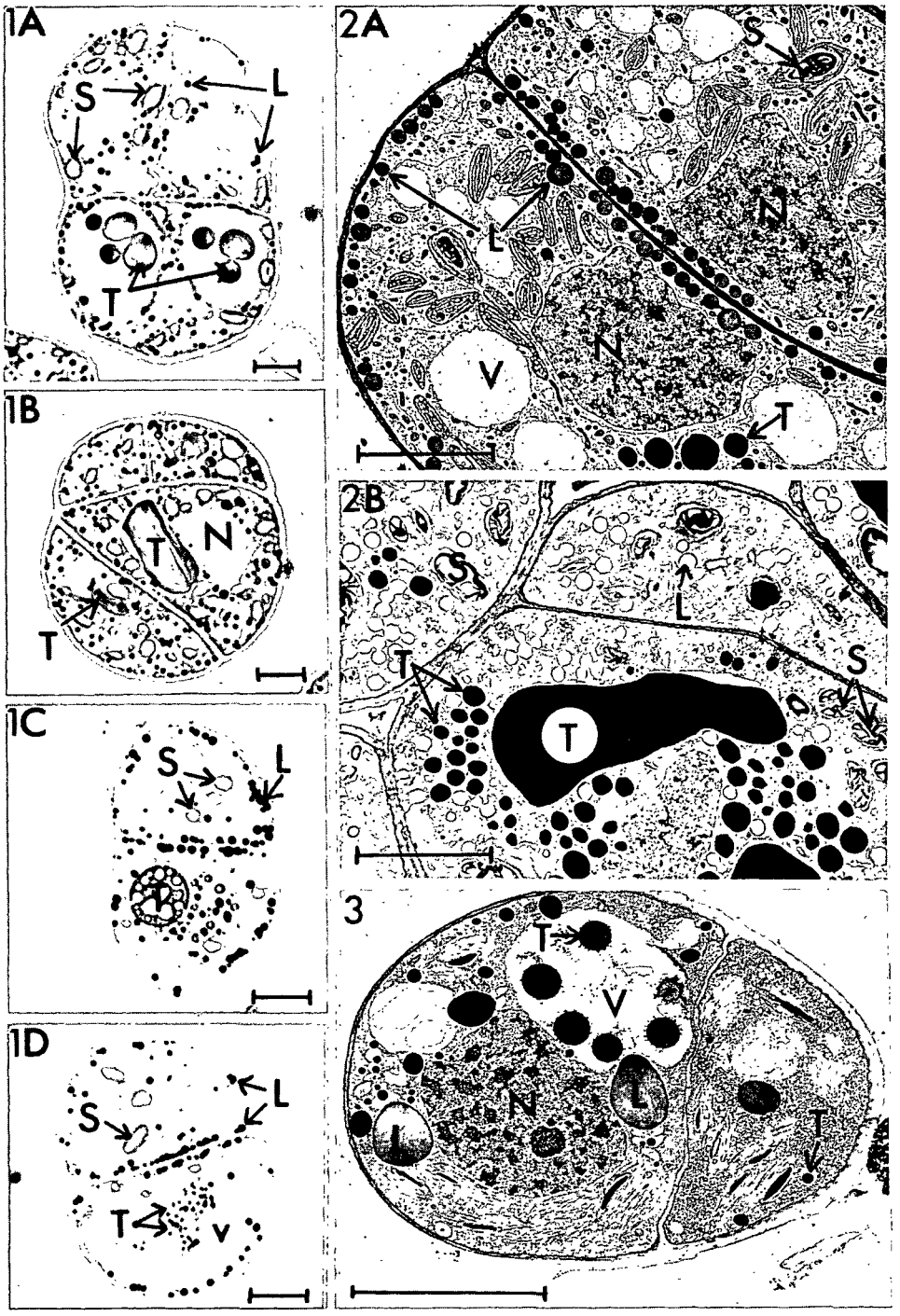
FIGURE CAPTIONS

Fig. 1A-1D. Optical light micrographs of semithin sections of callus cells from loblolly pine (Pinus taeda). A and B were taken in phase contrast, C in brightfield. T: tannin; V: vacuole; L: lipid body; S: starch grain in amyloplast; N: nucleus. The scale marker represents 10 µm in all micrographs.

Fig. 2A-2B. Transmission electron micrographs of ultrathin sections of the same callus type in Fig. 1. Note the gray lipid bodies and black tannin deposits.

Fig. 3. A cell doublet from callus of Douglas-fir (Pseudotsuga menziesii). Transmission electron micrograph. Both loblolly pine and Douglas-fir reacted identically to staining with Sudan Black B, all light micrographs being confirmed by electron microscopy of the same embedment areas.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